

Screening of metal-ion inducible genes from subseafloor sediments of Nankai Trough using substrate-induced gene expression method

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There are still large room for discovery in gene function and its potential of life in natural environment especially for the least-explored environments such as the subseafloor biosphere. We have employed substrate-induced gene expression (SIGEX) approach to screen DNA fragment that induce gene expression as a response to presence of substrates such as metal ions, D-amino acids, and persistent chemicals from metagenomic shotgun libraries prepared with subseafloor sediment samples of Nankai Trough.

Constructed metagenomics shotgun libraries that confers *gfp* gene downstream of the inserted DNA fragment were induced by metal ions including Ni²⁺, Co²⁺, Mg²⁺, Mn²⁺, Mo⁶⁺, and Ga³⁺, followed by sorting of clones with green fluorescence by a cell sorter. From sorted clones, we secondary screened clones for its response to the induction of each metal ion. Clones isolated by using Ni²⁺, Ga³⁺ showed specific response to each metal ion used for isolation. Also there were other type of clones that showed response to induction by multiple metal ions. DNA sequence analysis revealed that while around half of the clones had database-identifiable DNA fragment, other half did not show any match to DNA sequences in database. This study showed great potential of SIGEX-based approach to find function of DNA fragments from large pool on unknown environmental DNA of natural environment.

Keywords: Deep-sea sediment, Gene function, Nankai Trough, Metagenome, SIGEX, Metal-ion